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Dear Scientist,

The fifth International Congress on Advances in Bioscience and Biotechnology (icabb) was organized in Sarajevo, Bosnia and Herzegovina. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of veterinary area and to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country and mostly from Turkey. Total over the one hundred scientists were registered in the congress. The total number of submissions were 35 and after a careful evaluation 24 submissions were accepted by our scientific committee and 2 of them were accepted as poster presentation and 22 of them were accepted as oral presentation and all those presentations was taken place in the conference booklet.

We would like to send our special thanks to the International University of Sarajevo, Universiti Teknologi Malaysia, and Prof Hesham El Enshasy for their contributions. Also, we would like to express our special thanks to the organization team especially Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers, and the scientific committee. And finally, most importantly we thank all the participants individually to join this conference.

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INVITED SPEAKERS

Modern Nutrition: Advances in Systemic Biology as Current (Bio)Technological Approaches

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Introduction. The term of "modern nutrition" is currently almost equal to antiquity of the old testaments: "the food is medication and medication is food". And nowadays a lot of researchers are fascinated with concept of the "Healthy nutrition". Moreover, related to "microbiome" and omics studies suggest that this healthy nutrition suppose must need the individual requirements. Taking also into account the systemic biology and P4 novel medicine strategies visions we need to find a way of the integration and harmonization all our multifaceted knowledge in order to describe sufficiently and properly define the term of "personalised and healthy" nutrition – or by other words "modern nutrition"

Modern nutrition is a multidisciplinary and complex subject combining the approaches of the epidemiology, biochemistry, chemistry, behavioral science, biology, food science and medicine. Thus, we need to use all the available complementary data in order to construct "next generation functional foods".

Aim. The demands to these foods one might list: 1) be natural of origin, 2) be safe, 3) contains minimal or zero chemical / genetically modified additives, 4) be simple in preparation with minimal cooking efforts, 5) be fermented in order to be rich on biological active compounds and beneficial microbes 6) be prepared from naturally cultivated edible plants, 7) be clearly labeled of all the ingredients and 8) better be recognized by brand or at least by codes of food composition data bases.

Ideally will be to have a data about these products exact influence on human health, based on the results of clinical approbation: 1) of their different components or 2) whole meal / diets.

To meet this goal from first glance complicated (bio)technologies should be exploited. Interestingly that the majorities of these requirements are typical for the traditional well-known ethnical dishes that are 1) mostly accepted by nations, 2) can be easily prepared, 3) imagined as food with healthy impact on human health.

The challenges to make these new generation foods widely used are 1) short shelf life, 2) packing and transportation' difficulties, 3) varieties of recipes 4) (bio)technological gaps.

Results and Discussion. Recently we developed a line of such a novel traditional functional modern foods of new generation Ediens™. These products had been recently created particularly with unique microbial starters which are sequenced and preliminary selected / investigated in numerous in vitro, in vivo, ex vivo models.

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particularly with unique microbial starters which are sequenced and preliminary selected / investigated in numerous in vitro, in vivo, ex vivo models.

In addition, the synergetic properties of these strains of different phylogenetic groups with most popularly used plant originated biological active compounds isolated from local edible plants and berries had been carefully detected.

Person-specific efficacy to regulate human gut microbiome had been detected for individually prescribed foods via limited controlling diet studies.

Conclusion. In order to promote the implementation of newly developed functional foods relevant databases and AL for the calculation of personalized nutrition needs had been established and proposed.

Key words: Modern Nutrition, Food Composition Data Bases, Functional Food, Individual Nutrition, Human Microbiome, Databases, Al

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Climate Adaptation and Marine Biodiversity

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Climate change is the phenomenon of changing weather patterns and the inevitable increase of temperatures globally. While humans are primarily to blame for such a phenomenon, it will harm us and cause significant harm to every living creature on the planet. Climate change has and will directly negatively affect Biodiversity, which measures the number of species present in an area or globally and is a clear indicator of environmental health. It has been observed that increasing temperatures and altered weather conditions have been harmful to almost every organism. Thus, they are endangered, expected to decline in numbers and possibly become extinct. Unfortunately, the effects of climate change have already begun. The only thing we can do is collectively work together to help ourselves and our environment adapts to the expected changes. The key to doing so is innovation, science, and hard work. Biotechnology can allow us to make such adaptation measures and help us save our environment and its inhabitants. Many branches of this vast field of science exist, but genetics, particularly, has proven to be extremely valuable to biodiversity conservation. This particular field is promising because it deals with keeping organisms, or at least tissue fragments from them, in order to preserve them and keep their genetic material available for production in better survival circumstances to eliminate the risk of them going extinct. Biodiversity is a little talked about subject, its value severely underrated, and its true potential is yet to be unlocked. If we can save the remaining species, we open an unimaginable number of resources, pharmaceutical materials, biofuels, and much more goodness that can only be provided with a healthy, balanced environment.

Reverse Vaccinology: The Potential of Genome-Based Antigen Discovery and Vaccine Development

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Generally, vaccines are made of either killed or inactivated microorganisms. This traditional approach includes the growing of microorganisms, a laborious, expensive, and potentially hazardous process. With the advent of genome sequencing technologies, a novel approach for vaccine development has emerged, known as Reverse Vaccinology (RV). The principle of RV is to use the whole-genome sequence(s) of any microbe, including bacteria, parasites, or viruses, to identify the most potential vaccine candidate antigens without the need for cultivating the pathogen of interest. Identifying the candidate antigens is carried out by analyzing the genomic sequences *in silico*, using specialized computational methods and tools. The *in silico* discovery phase is typically followed by cloning, expressing, and evaluating the immune response of the shortlisted candidate antigens using *in vitro* and *in vivo* assays. In this talk, the basic principles of the original version of RV, which was developed almost two decades ago by Rino Rappuoli and co-workers to design a vaccine against serogroup B meningococcus, will be introduced. Furthermore, a group of different improvements that have been applied over the original RV design will be presented. In addition, some of the current opportunities and challenges facing biomedical scientists in using RV will be discussed.

Bio-Based Research Initiatives Directed Towards Building an African Bioeconomy

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A bio-economy covers all sectors and systems that are reliant on **biological resources and** their functions and is a new model for industry and the economy. It also exploits the untapped potential stored within millions of tons of biological waste and residual materials. The transition from a fossil fuel-based to a bio-based economy is expected to reduce the dependency on fossil fuels and achieve more sustainability as well as contribute to climate and environmental protection. In recent years, the bio-economy has also become a key focus of political and technological interest in various countries. Similarly, the vision for South Africa's bio-economy is to be a significant contributor to the country's economy by 2030 in terms of the gross domestic product (GDP). In order to achieve this, significant innovation is required for the development of novel industries that generate bio-based services and products. On a macro-economic and developmental level South Africa's thriving bio-economy has the potential to make the country more competitive internationally specially in the industrial and agricultural sectors. More importantly, a stable bio-economy will create more sustainable jobs, enhance food security and creates a greener economy as the country shifts towards a low-carbon economy. At the Council for Industrial Research (CSIR) a strategic research approach focusing on bio-based technology and product development has been adopted. Key platforms (Bio-conversions, Bio-based Products and Bio-manufacturing) have been established to fast track commercialisation and industrial uptake or for the establishment of new Biotech start-ups. An overview of these initiatives and successes will be presented.

ORAL PRESENTATIONS

The Impact of Facile Formulation of Alcohol-based Hand Sanitizer Containing *Eucheuma cottonii* Seaweed on its Sensory Properties and Acceptability

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Seaweed is a form of algae that be obtained abundantly from the sea. This rocky shoreline-growing alga is commonly eaten by the Asian people or used in culinary purposes. To further enhance the economic value of the seaweed, the applications need *cottonii* seaweed and to evaluate the sensory properties and overall acceptability. Different seaweed concentrations (1, 2 and 5% w/v) were screened for their suitability in the alcohol-based hand sanitizer formulation. Antimicrobial testing was also carried out on four common bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida utilis*) using Kirby-Bauer disk-diffusion method. A number of 30 volunteers consisting of men and women aged between 19-50 years old were given several samples of hand sanitizers including the commercial hand sanitizer and their evaluations were recorded and analyzed. It was observed that at higher seaweed concentration, the formulation becomes less viscous. The antimicrobial testing of the seaweed hand sanitizer formulation shows significant inhibition zones for all the four microbes tested to be diversified into high-value products. Seaweed have previously proven to have several properties including antimicrobial, thickening and moisturizing properties. With regard to the Covid-19 pandemic attacking the whole world, this study aims to develop a facile formulation of alcohol-based hand sanitizer containing Eucheuma with E. coli having the largest effect. Based on the overall acceptability, the seaweed formulation garnered mean score above 7 (like moderately) which indicates that the developed seaweed hand sanitizer has the potential to be accepted and commercialized.

Keywords: *Eucheuma cottonii*, seaweed, hand sanitizer, antimicrobial

Green technology influences the chemical composition of *Garcinia atroviridis* Extracts

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Many studies have focused on developing efficient and selective methods for extracting and isolating bioactive natural compounds. The value and quality of natural products in various applications has sparked interest in medicinal plant processing characteristics. This study aimed to improve the extraction of *Garcinia atroviridis* and characterise the chemical composition at different extraction times. Prior to the pre-treatment process, *Garcinia atroviridis* was extracted using a maceration method at intervals of 1 hour, 5 hours, 7 hours, and 9 hours. In this work, chemical quality control of the herbal extract was conducted by measuring polysaccharides, glycosaponin and protein. The results showed that the highest yield of extraction (35.2%) was obtained at 3 hours of extraction time. In this work, polysaccharide content was evaluated, and 0.37 % was recovered at the maximum extraction time of 5 hours. However, at 2 hours of extraction time, a greater protein content of 4.47 % was obtained, and at 9 hours of extraction time, a higher glycosaponin content of 62.2 % was gained. As a result, this maceration approach is a promising sustainable approach to enhance the potential of *Garcinia atroviridis* extract for future food and cosmetic applications.

Keywords: *Garcinia atroviridis*, maceration extraction, chemical composition

This research work is supported by HICOE Grant (4J272), Ministry of Higher Education Malaysia

In vitro performance of fatty acid-lipid nanocarrier as 5α-reductase inhibitors type II for hair growth promotion

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Androgenic alopecia (AGA) is one of the most common hair loss problems that affect both men and women during their lifetime. Due to the systematic side effects of oral finasteride, there is an increased interest in finding an alternative compound that can reduce hair loss problem but with fewer side-effects. Previous studies have shown that some plant extracts could inhibit 5α-reductase type II (5αR2) and due to the presence of fatty acids. In this study, fatty acid such as oleic and lauric acid were formulated into lipid nanocarrier (FA-LNC) for hair care cosmetic ingredients. However, up to now no specific fatty acid have been tested in vitro on human hair follicle dermal papilla cells to show cell proliferation and inhibition of 5αR2 activity. This research compares the effects lauric acid, C12 and oleic acid, C18:1 and also the performance of FA-LNC on the proliferation of hair follicle dermal papilla cells (HFDPc) and the inhibition the 5αR2 in vitro. FA-LNC with hydrodynamic diameter was found to be 230.1 ± 27.0 nm with a narrow PDI (0.270 ± 0.042). Oleic and lauric acid showed no cytotoxicity to the cell even at 1 mg/mL. At 0.0625 mg/mL, oleic acid showed significantly higher proliferation of the hair follicle dermal papilla cells compared to lauric acid ($p<0.05$). At 6.25%, FA-LNC showed better proliferation when compared to finasteride (positive control, 100 µM), oleic and lauric acid alone (1 mg/mL). In terms of the 5αR2 inhibition, at 0.0018 mg/mL, both fatty acids showed inhibition with no significant difference ($p>0.05$). Meanwhile, FA-LNC sample showed higher reduction activity compared to finasteride ($p<0.05$) with no significant difference with oleic and lauric acid alone ($p>0.05$). This study infers that FA-LNC as plant derived 5α-reductase inhibitors can act synergistically on the cell proliferation and have the potential to be added into hair growth products to inhibit the 5αR2.

Keywords: Androgenic alopecia, fatty acid, human hair follicle dermal papilla cells, 5αR2 activity

This study is supported by HICOE-IBD UTM (R.J130000.7809.4J317) and University Industry Research Laboratory UTM

New strategies for establishment of beneficial soil microbiological methods under ISO 17025

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Soil-beneficial microorganisms are important for biogeochemical cycles and have been used in agriculture production for decades. In the past years, agricultural practices have failed to promote healthy populations of microorganisms in soil, thus limiting the production yields and threatening sustainability. In this context, adequate monitoring of soil quality by a laboratory using analysis is needed as an indicator for the evaluation of soil quality. However, monitoring and evaluation can only be achieved if analytical methods are subject to an appropriate validation or audit process. The use of a verified method has become a prerequisite for a service laboratory certified to ISO 17025 (International Organization for Standardization) or other regulatory bodies in the country. In the framework of validation methods, the aim of this work is critically to discuss usual concept of regulatory guidelines used and common checklist that can be followed during the verification and validation work. Since nitrifying bacteria have been more prominent among beneficial microorganism analytical applications, the verification was done in accordance with standard method APHA-9245B by assessing the sensitivity, trueness, precision and robustness value of soil, wastewater and fertilizer samples. The method performance of sample analysis is well suited and was acceptable by the guideline with RSD value ≤ 0.1, coefficient of variation ≤ 10%, trainees value ranges from 60. 67%-127. 27% and robustness value 80-120% of both ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Further study needs to carry out in the validation of an alternative methods to demonstrate its capacity to perform as well as the gold standard reference method.

Keywords: Nitrifying, APHA 9245B, Soil, wastewater, fertilizer

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Bioprocess optimization for pleuran production by Pleurotus ostreatus using different cultivation strategies.

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Nowadays, large scale mushroom cultivation especially Pleurotus sp. takes attention of many pharmaceutical industries around the world due to their rich and variety of medicinal compound. Extensive research had been conducted to isolate, characterize and produce the importance bioactive compounds from this mushroom. Pleuran (β-(1,3/1,6)-D-glucan), is one of the important compounds belong to glucan group produced by Pleurotus ostreatus. This mushroom can be cultivated using submerged fermentation as a better alternative than the conventional method which used solid state fermentation. However, the production of this polysaccharide using submerged cultivation posed certain challenges due to its low yield. Therefore, this study aim to optimize the bioprocess conditions for high production of insoluble exopolysaccharide pleuran using submerged fermentation. Several media formulations were screened pleuran production. The best medium produced highest of 2.13 gL-1. Subsequently, the selected medium was optimezed using OFAT and statistical approached. By using statistical method, 3.65 gL-1 of pleuran was produced and this is higher compared to the OFAT method which produced only 3.33 gL-1 of pleuran. The optimized medium using statistical optimization was used in batch cultivation. The maximum pleuran production in batch cultivation was slightly higher than shake flask level at 3.75 gL-1. In fed-batch cultivation, full medium feeding was found to produce higher pleuran production of 4.5 gL-1 compared to mono carbon feeding with glucose produced 3.8 gL-1 of pleuran. In conclusion, this study has proposed a reliable approach for high production of plueran by Pleurotus ostreatus using the newly design medium and efficient cultivation strategy in the bioreactor.

Keywords: Bioprocess, submerged fermentation, fed-batch cultivation, pleuran

Study on bio accessibility and release behaviours of Gallic acid and Eurycomanone in herbal supplements products

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Herbal supplement product is a combination of the herbs with other ingredients has a variety of uses which allow them to bind together, regulate the release of the active compound and also upgrade the taste or hide the bitter taste. Bio accessibility is fraction or compound of active ingredients released from its matrix for intestinal absorption which will give impact on their bioavailability. Media solution and time are two factors which affect the behaviour of release of active ingredients. The objective of this study is to evaluate the release behaviour of the herbal supplements products submitted to different media using dissolution testing apparatus. In order to compare the release behaviour, four types of different media; 0.1 M HCl, 30% EtOH, acetate buffer (pH 4.8) and phosphate buffer pH 6.8 were used and samples were collected at 15 minutes time interval for duration of 105 minutes. The dissolution activity of the three brands of both Kacip Fatimah (I, II, III) and Tongkat Ali (TA, TB, TC) were compared. The aliquots sampling and active ingredients namely Galic acid and Eurycomanone in all samples were quantified using a reverse-phase HPLC. Pure standard of Kacip Fatimah (V) and Tongkat Ali (TS) powder were used as a benchmark. Results obtained indicate that the dissolution efficiency for active ingredients for all brands were varied, however amongst those, sample labelled as I in Kacip Fatimah and sample labelled as TA in Tongkat Ali showed the highest release rate of amount of galic acid and eurycomanone in an acidic medium (0.1 M HCl), respectively. Meanwhile, sample labelled as TC showed the lowest released rate of Eurycomanone in all medium tested. HPLC results showed that content of the Gallic acid in sample labelled as I was the highest while for content of Eurycomanone in sample labelled as TA was the highest. Complex mixture of ingredients which added in the Kacip Fatimah and Tongkat Ali products may give impact to the release behaviour of the active ingredients and affected by the solution media used.

Keywords: Herbal mixture, dissolution parameter, HPLC, quality

Extraction and Characterization of Astaxanthin From the Waste of Deep Water Pink Shrimp (P*arapenaeus longirostris*) Obtained from Iskenderun Bay

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The aim of this study is to purpose a simple and effective method for the isolation of high-value pigment astaxanthin from shrimp waste, a low-value raw material. In this study, acetone was used as an extraction solvent. Mobile phase for TLC was acetone: +hexane in the ratio 3:7 (v/v). The samples used in the study belong to the *Parapenaeus longirostris* species and were obtained from local fishermen operating in the Iskenderun Bay / Turkey. Shrimp waste was transported to the laboratory in a sterile container filled with ice. The wastes used in shrimp are the cephalothorax, abdominal shell and tail portion. Adhering meat from the cephalothorax was removed and the waste was washed under water and samples were dried at 50 °C with drying oven. Packed in polyethylene bags and stored at -18 °C until use. In this study, astaxanthin was extracted from shrimp shell waste using organic solvents (petroleum ether and acetone). Characterization of Astaxanthin pigment was performed with TLC buy comparing the Retardation Factor (Rf) as indicated in the Lorenz Todd standard chromatogram, in which three bands of Astaxanthin (Rf=0.36), Astaxanthin monoester (Rf=0.60) and Astaxanthin diester (Rf=0.75) were detected.

Keywords: Astaxanthin, *Parapenaeus longirostris*, thin layer chromotography

Assessment of Asthma-Associated miRNAs in Children with Rhinovirus Respiratory Infections

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Aim: Asthma is one of the major health problems all over the world. In fact, approximately two-thirds of asthma exacerbations are associated with human rhinovirus (HRV). MicroRNAs (miRNAs), small single-stranded, non-coding RNA molecules which are 22-25 nucleotides in length, are produced in cells. It has been reported that miRNA expression levels may be associated with the pathogenesis of diseases through the regulation of the production of target proteins. Expression changes of several miRNAs have also been found to play a role in the development and/or improvement of asthma. Still, relatively little is known about the role of miRNAs in asthma. We aimed to investigate serum levels of five asthma-associated miRNAs in children with lower respiratory tract infection (LRTI) caused by HRV compared to age-matched healthy controls.

Materials and Method: Serum samples were collected from the 37 pediatric patients, aged 0-3 years, with LRTI proven to be caused by HRV using RT-PCR assay (FTD Respiratory pathogens 21, Fast Track Diagnostics, Luxembourg) and from the 30 age-matched healthy controls. miRNA let-7a-5p, miR-9-5p, and miR-155-5p were investigated from the serum samples by using RT-PCR assay (RTA miRNA Kit, Kocaeli, Turkey). miRNA serum expression levels were analyzed by the 2(−ΔΔCT) method.

Results: In the present study, we found that let-7a-5p, miR-9-5p and miR-155-5p levels were significantly increased, while miR-146a-5p and miR-19-3p levels were significantly decreased in the sera of children with HRV infection compared to the age-matched control group. Assessment of serum expression changes of asthma-associated miRNAs in children with respiratory tract infections caused by HRV is important for understanding the effect of HRV on the pathogenesis of asthma through miRNA expression changes. On the other hand, longitudinal studies based on clinical observation are needed to determine the association of HRV infection with the development of asthma and miRNA-induced epigenetic effects."

Keywords: Human rhinovirus, asthma, miRNA let-7a-5p, miR-9-5p, miR-146a-5p, miR-19-3p, miR-155-5p

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The Investigation of the Therapeutic Effects of Epigallocatechin-3-Gallate Against Cisplatin-Induced Liver Damage in Rats

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In this study, it is aimed to investigate the possible protective effects of epigallocatechin-3 gallate (EGCG) in rats exposed liver damage with cisplatin. The animal experiments part of this study was conducted in the F.U Experimental Animal Research Center (FUDAM) with the permission of the F.U Animal Experiments Ethics Committee dated 17.02.2021 and numbered 2021/03. In the study, 28 Wistar albino male rats (n = 28, 8 weeks old) were divided into 4 groups and each group included 7 rats. Groups: (i) Control Group: Standard diet, (ii) EGCG Group: Standard diet + EGCG (50 mg/kg CA, ip), (iii) Cisplatin (CP) Group: Standard diet + Cisplatin (CP) (7 mg/kg CA), (iv) EGCG + Cisplatin (CP) Group: Standard diet + Cisplatin (CP) (7 mg/kg CA) + EGCG (50 mg/kg CA, ip). The rats were decapitated after 4 weeks and their liver tissues were taken and examined. Expression levels of caspase-3 and Bcl-2 proteins in liver tissue were determined by western blotting technique, lipid peroxidation MDA (malondialdehyde) analysis, catalase and GSH (glutathione) levels were determined by spectrophotometer. Compared to the Bcl-2 protein expression and malondialdehyde (MDA) level decreased, caspase-3 protein expression level, glutathione (GSH) level and catalase activity (CAT) were significantly increased in the groups EGCG + CP compared to the group CP. As a result of this study, it was determined that EGCG treatment has protective and therapeutic effects against liver damage.

Keywords: EGCG, liver injury, cisplatin, MDA

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The Effect of Fulleren C60 Nanoparticle on Caspase-3, Bcl-2, Nrf-2, NF-κB, TNF-α, Cox-2, p53, IL-6, IL-1α and MAPK Protein Signal Pathways Against Breast Cancer Exposed by DMBA (7,12-Dimethylbenz [a] Anthracene) in Rats

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In this study, the anticancer effect of C60 nanoparticle against breast cancer caused by DMBA (7,12-dimethylbenz [a] anthracene) in Wistar albino female rats was investigated. The animal experiments part of this study was conducted in the F.U Experimental Animal Research Center (FUDAM) with the permission of the F.U Animal Experiments Ethics Committee dated 13.02.2019 and numbered 2019/03. In this study, 60 Wistar albino female rats (n = 60, 8 weeks old) were used. These rats were divided into 4 groups and each group included 15 rats. Groups: (1) Control Group: Fed with standard diet; (2) Group C60: C60 (1.7 mg/kg bw, oral gavage); (3) DMBA Group: DMBA (45 mg/kg bw, oral gavage); (4) C60 and DMBA Group: C60 (1.7 mg/kg bw, oral gavage) and DMBA (45 mg/kg bw, oral gavage) group. The rats were decapitated after 16 weeks and their breast tissues were taken and examined. Expression levels of caspase-3, Bcl-2, Nrf-2, NF-κB, TNF-α, Cox-2, p53, IL-6, IL-1α and MAPK proteins in breast tissue were determined by western blotting technique. As a result, Bcl-2, NF-κB, TNF-α, Cox-2, IL-6, IL-1α and MAPK protein expression levels decreased, caspase-3, Nrf-2 and p53 protein expression levels were significantly increased in the groups C60 + DMBA compared to the group DMBA. According to the results obtained from this study, it suggests that the C60 nanoparticle will make significant contributions to the development of new drugs for breast cancer treatment.

Keywords: Apoptosis, Bcl-2, breast cancer, caspase-3, fulleren C60, Nrf-2, NF-κB

Acknowledgement: This work was supported by Firat University Scientific Research Projects Unit (FUBAP) with FF. 20.07 project number. In addition, this study was supported by the Council of Higher Education (CoHE) 100/2000 Biotechnology priority field doctoral project and The Scientific and Technological Research Council of Turkey (TUBITAK) 2211/C program.

An investigation on thiol/disulphide homeostasis on cattle naturally infected with bovine ephemeral fever

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Three-day sickness or bovine ephemeral fever is an acute febrile viral disease instead of cattle and ruminant species on Middle East, Asia, Africa and Australia. The aim of this study was to clarify the thiol/disulphide homeostasis on cows naturally infected with bovine ephemeral fever. For this propose total of 45 cows were enrolled to study and divided in to three equal (n=15) groups. Cows were clinically infected, recovered and healthy were determined as Group I, Group II and Group III, respectively. Blood samples were taken without attempting to treat any of the animals in the groups. The native thiol levels were found to be significantly lower in Group I (262,9 ± 25,1) and Group II (253,1 ± 39,1) compared to Group III (300,8 ± 52,4). Non-significantly reduces were observed on Total thiol levels were also detected in Group I (339,1 ± 23,9) and Group II (242,5 ± 40,3) compared to Group III (367,1 ± 55,6). Disulphide levels, disulphide/total thiol, native thiol/ total thiol and disulphide/native thiol ratios were similar in all the groups (P>0.05). In conclusion, bovine ephemeral disease in cows was affected by the thiol disulphide hemostasis. This is the first study to determine the thiol/disulphide hemeostasis in cows with bovine ephemeral fever. Therefore, the results achieved in this research might be ground for further studies to include antioxidants in treatment practices.

Keywords: Thiol/disulphide homeostasis, ephemeral fever, cattle

Corona Virus and Our Environment

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Corona virus disease (COVID 19) is a global pandemic, affecting human life severely. It has infected 181.5 million people world-wide which resulted into death of 3.9 million (WHO 2021). In April and May 2021 sudden surge in cases of corona virus infection was reported from different parts of India. Many scummed to death while those who somehow survived from COVID 19 tsunami has suffered from post-covid symptoms. Considering the scale of its impact, it is important to investigate the public opinion about the spread and origin of corona virus. As COVID is a zoonotic disease we therefore asked public whether there is any linkage between environment and spread of corona virus? To assess the public opinion, we conducted an online survey with the help of undergraduate students of the Shivaji College during June-July, 2021. An online questionnaire on 'Google forms' was prepared and circulated by students through social media and email. A total of 1260 individuals participated in the survey. Based on the survey we found that most of the participants were aware about the causative organism of COVID 19. Majority of them agreed that there is a linkage between degradation of environment and spread of corona virus. They also believe that deforestation, wildlife trade, hunting, landuse change have played role in spread of zoonotic diseases. They further believed that spread of such diseases would likely to aggravate under climate change. Majority of them agreed that vaccination along with conservation of biodiversity, prevention of deforestation landuse change, prohibiting wildlife trade and human wildlife conflict could effectively prevent the spread of zoonotic diseases such a COVID 19. However, around one-fourth of them were of neutral opinion and believed evidences and facts published in print and online media were inconclusive. The current study though presented mixed response from Indian public, however higher proportion of individuals believed a strong linkage between environment and corona virus spread.

Keywords: Corona virus, zoonotic diseases, survey, public perception, India

A Novel Formulation for a Probiotic Supplemented Confectionery with Natural Ingredients

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Probiotics are living organisms that have beneficial effects on host by regulating the microbial balance of the intestinal system. Although mostly in dairy products, it is possible to see examples of food products supplemented by probiotics in bakeries, chocolates and confectioneries. Nowadays, the COVID-19 pandemic that the world suffers increased the demand for such functional food products including probiotics. Due to probiotics having potential effects on strengthening the immune system, a novel formulation for a probiotic supplemented confectionery with natural ingredients is proposed together with a market research to compare the benefits of similar products. The formulation with natural ingredients in the form of jelly beans supplemented by probiotics in the same package suggests varying potential benefits specifically focusing on the probiotics including Lactobacillus species. In this study, a sensory evaluation was carried out to compare the consumer demand for the jelly formulation of the product. 35 untrained panelists were given 4 different samples with varying ratios of carrot and spinach together with a fixed ratio of lemon juice and apple sugar. The panelists were first asked the questions that would determine the general consumption trends and then the parameters including willing to buy, smell, appearance, taste, sweetness, color, texture, shape, bitterness, aftertaste, acidity, overall acceptability were asked to be evaluated in a 7-point scale with 9 questions. Accordingly, an average score of over 4 was achieved with the novel formulation for the confectionery supplemented by probiotics as follows (for 100 g of the product): 31 g of carrot, 7 g of gelatin, 10 g of apple sugar and 0.72 g of lemon juice and 51 g water. These amounts meet 13.2% of protein, 1.23% of carbohydrate, 2.5% of water, 3.3% of sugar, 7.9% of fiber, 11.6% of Vitamins A and C, 0.1% of fat. In addition, the probiotic content reaching over log 7 cfu/mL is suggested to meet 100% of the daily needs. In conclusion, it was depicted that production of a probiotic confectionery containing vegetables or fruits would be useful to help fight several diseases and reach a stronger health level. In the future, this product can be modified for both probiotic bacteria content and flavor in various foods in accordance with consumer feedbacks. The outcomes provide a guide together with the recommendations for potential probiotic research in candy and confectionery industry.

Keywords: Probiotics; confectionery; natural ingredients; lemon juice; carrot

Acknowledgements: The authors gratefully acknowledge Food Engineer Eylül Naz Aktaş for assistance with the sensory analysis.

Ethics approval: Ethics approval and consent to participate he questionnaire and methodology for this study was approved by the Science and Engineering Research Ethics committee of Izmir University of Economics (Ethics approval number: B.30.2.IEOFMB.0.05.05-020-040)

Investigation of novel industrial proteins from different source by metagenomic approaches

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The discovery of novel enzymes from nature has attracted attention due to the benefits of enzymes in industrial synthesis technology. Catalysis with enzymes offers several advantages over chemical catalysis. Enzymes improve manufacturing processes, decrease energy usage, work under mild reaction conditions, reduce hazardous by-products, and increase substrate specificity. Enzymes are also biodegradable thus offer an environmentally friendly technology. There are still many types of microorganisms that are not explored or identified yet, to be a novel enzyme source.

Since less than 1% of total microorganisms in nature can be cultured in the laboratory, a combined approach has been adopted for new protein mining, which includes the functional screening of a microbial resource and a metagenomic library. Metagenomics is a strategy used to analyze whole microbial genomes by extracting total DNA directly from environmental samples without culturing. It is important to discover new proteins that can be used to develop new biotechnological approaches. In our studies, we perform metagenomic studies to determine the microbial community in different environments and to obtain new enzymes. Metagenomic libraries that we obtained from different sources such as rich humus soils, butcher's wastes buried soils, acidic and halophilic food fermentation environments, and unusual cave environments continue to be screened for their industrial potential with sequence-based and functional-based approaches. The determination of microbial diversity in these environments have been completed. Enzymatic activity has been screening according to the culture-dependent and independent approaches. As a result of these studies novel enzymes with glucanase, lipase, and dehydrogenase activity were isolated and recombinantly expressed. Optimization of heterologous expression in different hosts and, biochemical and thermodynamic characterization studies of enzymes in different pH, temperature, salt concentration and organic solvent are in progress."

Keywords: Metagenomics, microbial diversity, novel enzymes, biotechnological production

Effect of dietary taurine supplementation on growth performance of juveniles shibbot (*Tor grypus*)

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This study was conducted to evaluate the effects of taurine on growth performance of Shibbot fish (*Tor grypus*). In the study, 0.00% (C), 0.5% (G1), 1.0% (G2) and 1.5% (G3) taurine were added to the bait of Sabut fish and fed for 60 days. The effects of Taurine on the growth parameters of the Shibbot fish and feed evaluation and aquaculture economy were investigated. Live weight gain (WG), feed intake (FI), Feed conversion rate (FCR), Protein efficiency ratio (PER), Specific growth rate (SGR), Survival rate, Economic conversion rate (ECR) and its effects on the economic profit index (EPI) were investigated. At the end of the study, Sabbut juveniles with an initial weight of 2.28 ± g reached 3.11 ± 0.16 g, 3.05 ± 0.17 g, 3.25 ± 0.11 g, and 3.14 ± 0.26 g respectively. It has been observed that the addition of taurine at the rate of 1% has a positive effect on FCR value in breeding of Shibbot fish and other trails have reached similar and higher FCR values. Considering the contribution of taurine in terms of aquaculture economy, it was also determined that the G2 allows production at a lower cost (USD / kg). It has been observed that adding taurine to Shibbot fish feeds does not contribute to EPI value (economic profit index).

Keywords: **T**aurine, aquaculture, shibbot fish.

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Extracellular keratinases of *Cladosporium sphaerospermum* for biodegradation

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Animal husbandry produces enormous amounts of by-products that need to be disposed of, e.g., wool, feathers, and bristle. A major structural component of these wastes is keratin - a rigid fibrillar protein with a protection function that is hard to degrade. Enzymatic biodegradation is a beneficial and green approach to keratin-waste disposal. A few organisms groups are capable of producing keratinases - enzymes that break down keratin - including micromycetes. For a filamentous fungus Cladosporium sphaerospermum, we showed inducible keratinolytic activity in alkaline conditions in previous studies, but for biotechnological application, a proper study is essential.

For further investigations, submerged fermentation of C. sphaerospermum was performed in a previously selected nutrient medium that differs in keratin sources inducing enzyme synthesis (ground chicken feathers, purified keratin from wool) and pH (8, 9). General proteolytic and keratinolytic activities were determined with culture liquid with 1% suspensions of casein and keratin (prepared on 0.05M Tris-HCl buffer, pH 8.2) respectively and measured spectrophotometrically. One unit (U) of activity was defined as an increase of absorbance by 0.01 under the assay conditions (37 °C).

The highest general proteolytic activity was measured with a sample obtained on the 3-rd day of cultivation on a medium with purified keratin (pH 8), representing 77.3 U. The highest keratinolytic activity was registered after three days of cultivation with ground chicken feathers (pH 9), reaching 45.0 U. Moreover, it remained stable, slightly decreasing to 42.3 U by the seventh day of cultivation.

Thus, for C. sphaerospermum, an intense and stable synthesis of keratinases observed when growing on feathers illustrates the potential of C. sphaerospermum for biodegradation of poultry waste.

Keywords: Keratinases, micromycetes, biotechnology, biodegradation

Histology of Digestive System in the Nannospalax xanthodon (Rodentia: Spalacidae)

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Blind mole rats (Nannospalax xanthodon) are subterranean rodents with various morphological and physiological adaptations to underground life. The aim of the present study was to reveal histological structure of the digestive system of the N. xanthodon which usually prefers a foods such as plant root, tubers and bulbs. For this purpose, five blind mole rat of both sex were used for this study. Tissue samples of the digestive tract were kept in 10% neutral buffered formalin, processed using routine histological methods, and mounted in paraffin blocks. Six micrometer-thick sections were cut and stained with Crossman's trichrome, H&E, alcian blue (pH 2.5) and periodic acid Schiff method.

The results of this study have shown that the esophagus of N. xanthodon is lined by stratified squamous keratinized epithelium with no lamina muscularis and glands. The stomach of this blind mole rat is unilocular compound type. The glandular region contains simple tubular gland, that is lined by mucous neck cells, chief cells and parietal cells. Intestinal glands (crypts) are observed in the lamina propria layer of the duodenum. The length of the villus intestinalis was shortened in the ileum compared to the duodenum, and the number of goblet cells increased considerably. Aggregated lymphoid follicles were observed in the lamina propria and submucosa layers. Tunica muscularis layer was thicker in colon and rectum. Histologically, it was observed that the liver consisted of polygonal shaped lobules. It was determined that the interlobular interstitium was not prominent around the lobules. Some of the polygonal shaped hepatocytes were found to be binucleated. The wall of the gallbladder (vesica fellea) is formed as four layers, inward to outward: lamina epithelialis, lamina propria, tunika muscularis, and serosa. The lumen of the vesica fellea is lined with a high columnar epithelium. Tunica muscularis is a narrow layer of smooth muscle cells and dense collagen and elastic fibers.

In conclusion, although the digestive system of blind mole rat is histologically similar to other rodents, it was observed in the present study that it has also some specific features of its own. These findings are important in having a better understanding of their feeding and adaptation to underground life.

Keywords: Histology, digestive system, *Nannospalax xanthodon*

Using quantile regression approach to evaluate the effects of some selected parameters on daily milk yield in Holstein cows

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Milk yield is an important parameter for the dairy products industry. Days in milk, lactation number and the number of insemination are also reproductive factors that affect milk yield. In the field of veterinary medicine, the effects of these factors (independent variables) on milk yield (dependent variables) can be determined using regression models. The aim of this study was to evaluate days in milk, the number of lactation and the number of insemination that affect the daily milk yield in Holstein cows with quantile regression approach.

The material of the study consisted of the yield records of 112 healthy and multiparious holstein cows. The cows were kept in a private farm and had no dystocia. The effects of days in milk, lactation number and the number of insemination on daily milk yield were examined by linear regression and quantile regression models. First, the distribution of error terms, outliers and influential cases (Mahalanobis distance) were determined for the suitability of linear regression. The quantile regression model, which is an alternative approach to the linear regression method, was applied to determine the parameters that may affect daily milk yield.

As a result of statistical analysis, it was determined that the error terms in linear regression did not fit the normal distribution. Also, there were influential cases according to the Mahalanobis distance. In the quantile regression model, which is a flexible approach compared to linear regression, 0.25th, 0.50th and 0.75th quantiles were examined. It was observed that the number of insemination in 0.25th quantile had an important effect on daily milk yield ($p<0.05$), while days in milk ($p>0.05$) and number of lactation ($p>0.05$) were insignificant. In 0.50th and 0.75th quantiles, the parameters included in the model were not statistically significant.

As a result, the number of insemination had a effect on daily milk yield in only 0.25th quantile. In addition, the quantile regression model can be used as an alternative method when the assumptions are not met in linear regression.

Keywords: Milk yield; quantile regression; reproductive parameters

Determination of β-Glucosidase deficiency by using in house ELISA model

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Rare diseases (RD) refer to diseases seen less than 1:2000. There are about 8000 species of RD of which 80% are of genetic origin. In recent years, lysosomal storage diseases, which belongs to RD family, (LSD; most commonly seen species such as Gaucher) are of enhanced importance all over the world, seem a lack of a specific enzyme/lysosomal component in the body. According to the Health Implementation Communiqué, these diseases are diagnosed according to the measurement of enzyme level or mutation analysis results are compatible with the RD.

In this study; we tested novel conjugate systems to being developed the sensitivity of antibodies for β-Glucosidase level by using sandwich ELISA method. We coated the plates with monoclonal anti-GBA (1 µg/mL in Glycerol 40%) and Anti-GBA antibody (0,5 µg/mL in Glycerol 40%). Then we compared free conjugate and hybrid conjugates. We tested different concentration (1,56-100 ng/mL) of GBA enzyme standards with covered plates. We accepted the cut-off value between the highest value of the negative controls and the lowest value of the positive controls (2 x mean of negatives). In that sense, only the formulas correctly discriminated amongst the positive and the negative controls. Our study evaluated the diagnostic accuracy of β-Glucosidase protein level. This aims to eliminate and alleviate the socio-economic burdens of RD, to diagnose easily and correctly, transition to rapid treatment or to develop information production and methods that can be effective in preventing diseases.

Keywords: ELISA, Gaucher Disease, diagnostic System

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POSTER PRESENTATIONS

Vermiculite as a new carrier for fungal proteases production in solid-state fermentation conditions

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Micromycetes are known as producers of extracellular proteases with broad substrate specificity. An efficient approach to increase the hydrolases yield for biotechnology is using solid-state fermentation (SSF). SSF provides overproduction of enzymes because fungal mycelium penetrates and entwines the carrier particles, imitating natural growth conditions. Besides, some enzymes are synthesised only during solid-state but not submerged fermentation. Therefore, carrier selection plays an essential role in enzyme production for industrial applications.

As high-active protease producers, Aspergillus ochraceus L-1 and Aspergillus ustus 1 were selected for the study. Strains were cultivated stationary at 28 °C on polyurethane foam, perlite, silica gel, and vermiculite, moisturized with a previously selected nutrition medium. Proteolytic enzymes were eluted on the 5-th day of cultivation with 0.05 M Tris-HCl buffer, pH 8.2. The general proteolytic and fibrinolytic activities were determined using 1% suspensions of casein and bovine fibrin. Proteolysis was carried out with eluted enzymes and measured spectrophotometrically. The activity was expressed in μmoles of tyrosine formed in 1 min in 1 mL of culture liquid and was calculated using the calibration curve. For productivity, the obtained result was divided by the mass of dry mycelium, previously determined gravimetrically.

Compared with silica gel, the yield of proteinases on vermiculite was 3 times higher than other carriers - from 7 to 18 times. This proved polyurethane foam and perlite as ineffective carriers for SSF. Proteolytic activity of both micromycetes was the highest when growing on vermiculite (52.4 and 57.2 U/mg of biomass 10^-3 for A. ochraceus L-1 and A. ustus 1, respectively). Also, for A. ustus 1, SSF induces the production of fibrinolytic proteases, with the highest activity (224.4 U/mL) observed with vermiculite.

Thus, vermiculite may be considered a suitable carrier for SSF, increasing producers' productivity and yield of enzymes for biotechnology.

Keywords: Solid-State Fermentation, Vermiculite, Proteases

Effects of mitochondrial-derived peptide (mots-c) on cell death and dna damage of mcf-7 breast cancer cells

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Breast cancer is an important health problem among women. The discovery of new therapeutics to be used in cancer treatment is important for increasing success in treatment. Mitochondrial-derived peptide (MOTS-c) is encoded by mitochondrial DNA and is 16 amino acids long. MOTS-c is involved in cellular energy metabolism by activating 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)-mediated AMP-activated protein kinase (AMPK).

The present study aimed to determine the cytotoxic and genotoxic effects of MOTS-c on the human breast cancer cell line, MCF-7.

In the present study, MCF-7 cell line was used and the cells were cultured with DMEM medium. After treatment with MOTS-c and the standard anti-cancer drug 5-fluorouracil (5-FU), the cytotoxicity level in the cells was determined by MTT assay, and the genotoxicity level was determined by Comet assay. Furthermore, mRNA and protein expression levels of genes involved in cell death processes were investigated using qPCR and Western Blot, respectively.

The results showed the cytotoxic effect of MOTS-c on MCF-7 cells was more pronounced at 72 hours. In Comet analysis of MCF-7 cells after MOTS-c application, tail intensity (TI), tail lenght (TL), and tail moment (TM) parameters were significantly increased compared to control group ($p<0.05$). MOTS-c application caused a significant decrease in Akt and Bcl-2, while an increase in AMPK, Beclin1, p53, TSC2, and ULK1 expressions in MCF-7 cell line($p<0.05$).

These results are the first data showing that MOTS-c causes cell death through DNA damage in MCF-7 cells. In conclusion, MOTS-c may cause an increase in AMPK-mediated TSC2 and ULK1 gene expressions and thus can activate the autophagy pathway in cancer cells.

Acknowledgment: This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: 318S235).

Keywords: 5-Fluorouracil, Apoptosis, Cytotoxicity, Genotoxicity, MCF-7, MOTS-C

FULL TEXTS

ŞABUT (*Tor grypus*) BALIĞININ TAURİN DESTEKLİ YEMLERLE BESLENMESİNİN BÜYÜME PERFORMANSI ÜZERİNE ETKİLERİ

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ÖZET

Çalışmada yarı esansiyel bir amino asit olan Taurinin şabut balığı yavrularının büyüme parametreleri, yem değerlendirme verileri ve yetiştiricilik ekonomisi üzerine etkileri araştırılmıştır. Bu amaçla yemlerine %0,00, %0,5, %1,0 ve %1,5 oranlarında taurin ilave edilen yavrular, 60 gün boyunca fiber tanklarda yetiştirilmiştir. Denemede %32 ham protein içerikli ticari sazan yemi kullanılmış, yemleme elle yapılmış ve balıkların canlı ağırlıklarının %3 ü oranında verilmiştir. Araştırmada büyüme parametreleri olarak canlı ağırlık kazancı (CAK), Yem değerlendirme oranı (FCR), Spesifik büyüme oranı (SGR), Hayatta kalma oranı, Ekonomik dönüşüm oranı (ECR) ve Ekonomik yarar indeksi (EPI) araştırılmıştır. Taurin destekli beslemenin şabut yavrularının büyümeleri üzerine etki pozitif etki etmemiştir. Başlangıç ağırlığı 2,28±0,01 g olan Şabut balığı araştırma sonunda sırasıyla 3,11±0.16 g, 3.05±0.17 g, 3.25±0.11 g, ve 3.14±0.26 g ağırlık ortalamalarına ulaşmıştır. Taurinin şabut balığı yavrularının yem değerlendirme oranı üzerine olumlu bir katkı yaptığı gözlenirken, en iyi oranın G2 (4,65±0,86) de elde edildiği saptanmıştır. Benzer bir biçimde, % 1 oranında taurin eklenmesinin ECR değerini olumlu yönde etkilediği ve üretim maliyetini düşürdüğü görülmüştür. Bunların yanı sıra taurin eklenmesinin şabut balığı yavrularının SGR, Oransal büyüme, EPİ ve Hayatta kalma oranı değerleri üzerine etki etmediği bulunmuştur.

Anahtar Kelimeler: Taurin, Balık Yetiştiriciliği, Şabut balığı

ABSTRACT

Taurine, which is a semi-essential amino acid, is added to the feed of Shibbot juveniles (0.00% (K), 0.5% (G1), 1.0% (G2) and 1.5% (G3)) regarding growth parameters, feed conversion ratios, aquaculture economics. Effects on the data were investigated. The experiment was carried out in fiber tanks for 60 days. In the experiment, commercial carp food with 32% crude protein content was used, the feeding was made by hand and 3% of the body weight of the fish was given. Live weight gain (CAK), feed intake (YA), feed conversion ratio (LF), protein efficiency ratio (PEO), specific growth rate (SBO), survival rate, economic conversion rate (ECR) and the effects on the economic benefit index (EYI) were investigated. Total raw protein, lipid, crude ash and moisture content were calculated as nutrient content. At the end of the study, shout fish, which had an initial weight of 2.28 ± g, reached 3.13 ± 0.16 g, 3.05 ± 0.17 g, 3.25 ± 0.11 g, and 3.14 ± 0.26 g, respectively. Continuous observations were made during the daily feeding process and we decided to carry out a second trial after weighing the results of this weak growth every 2 weeks. This time, we did not use taurine, we wanted to eliminate the stress factor and observe what would happen as a result. By reducing the stress factor on 3 different groups, we would get results with 3 different feeds. 1. Group of plain carp feed; 2. Guba carp and trout feed mix; Group 3 was given simple trout feed and weighing was not performed to

reduce stress factor for 45 days. In the second trial the initial weights were 2.38 g on average and the trial lasted 45 days. At the end of 45 days, fish fed with carp feed had an average weight of 3.42 ± 0.16 g, fish fed with mixed feed was 4.17 ± 0.06 g and fish fed with trout feed had an average weight of 4.50 ± 0.02 g. In the study, daily average feed consumption was kept constant as 3% of the individual weight.

Keywords: Taurine, aquaculture, shibbot fish.

GİRİŞ

Türkiye'de ve Dünya'da son yıllarda özellikle su ürünleri üretimi ve tüketimi alanında önemli gelişmeler gözlenmektedir. Su ürünleri sektörü dünyada en hızlı büyüyen sektörler arasında gelmektedir. Gerek sahip olunan geniş doğal kaynaklar, gerekse teknik, ekonomik ve sosyal yaşamdaki ilerlemeler sektörün gelişmesine etki eden faktörlerdir. Ülke nüfusunun hayvansal protein açığının kapatılmasında, yeterli ve dengeli beslenme düzeyine erişilmesinde su ürünleri son derece önemli bir yere sahiptir. Entansif koşullarda balık yetiştiriciliğinde amaç; ekonomik koşullarla en kısa sürede balıkların istenilen düzeye getirilmesidir. Bunu gerçekleştirebilmek için de uygun şekilde hazırlanmış yemlerle balıkların yeterli bir şekilde beslenmesi gerekmektedir (Keskin ve Erdem, 2005; Öz, 2016).

Şabut balığı, Dünyada ve Türkiye'de üretimi ve yetiştiriciliği pek olmayan kültür balıkları arasında olmakla birlikte yapılacak geliştirme çalışmaları ile Türkiye su ürünleri ihracatı içerisinde önemli bir yere sahip olabilir. Ayrıca Fırat ve Dicle nehirlerinde yaşayan bir balık olup bölge halkı tarafından tutulan bir balıktır. Yahudiler tarafından da kutsal balık olarak tabir edilirler.

Taurin, hayvan görme sisteminde önemli bir nörokimyasal faktördür (Omura ve Yoshimura 1999; Militante ve Lombardini 2002). Hem insanda hem de hayvanlarda görsel işlev bozukluğu, taurin eksikliğinden kaynaklanabilir (Militante ve Lombardini 2002). Bu eksiklik eksojen taurin takviyesi ile tersine çevrilebilir. Taurinin, balıklarda retina gelişimi ve görsel sistem üzerindeki rolü üzerine birtakım çalışmalar yapılmıştır. Bol taurinin, retina fotoreseptöründe ve yavru pisi balığı (Omura ve Yoshimura 1999) ve akvaryum balıklarının sinir tabakalarında lokalize olduğu bildirilmiştir (Nusetti ve ark. 2009). Bu sonuçlar, taurinin, fotoreseptör dış bölümünün korunmasında, nöral iletim düzeninde ve gelişim evrelerinde fotoreseptör farklılaşmasında rol oynayabileceğini göstermektedir. Ayrıca, (Nusetti ve ark. (2009) taurin taşıyıcı, taurin ve çinkonun da fotoreseptörlerde ve ganglion hücre katmanında bir arada bulunduğunu ortaya koymuş hem taurinin hem de çinkonun balık retinasında normal hücresel fonksiyonlarda önemli olduğunu belirtmişlerdir.

Bitkisel kökenli bileşenlerin çoğu kükürt içeren amino asitlerin metabolizmasının bir son ürünü olan taurin (2 - aminoetansülfonik asit) ile de sınırlıdır. Taurin, bir karboksil grubuna sahip olmamasına rağmen, genellikle amino asit olarak sınıflandırılır. Aynı zamanda, memeli dokularının protein sentezine veya bozulmasına da dahil değildir (Kuzmina *ve* ark. 2010). Bununla birlikte, taurin, hayvan türüne bağlı olarak bütün amino asit havuzunu % 30-50'sini oluşturur (Jacobsen ve Smith, 1968). Taurin, memelilerde, immün yanıtın modülasyonu, kalsiyum taşınması (Takahashi *ve* ark. 1992), retina gelişimi (Omura ve Yoshimura, 1999) dahil

olmak üzere birçok fizyolojik fonksiyonda rol oynar. Safra asidi metabolizması (Hofmann ve Small 1967), osmotik regülasyon (Thurston ve *ark*. 1980) ve endokrin fonksiyonlarının (Kuzmina *ve* ark. 2010).

Birçok balık türü için hazırlanan rasyonlarda olduğu gibi alabalık yem rasyonlarının da önemli bir kısmını balık unu oluşturmaktadır. Balık ununa bağlı olarak yem fiyatları sürekli arttığından sektördeki büyümeyi sürdürülebilir hale getirmek gerekmektedir. Yetiştiricilik sektöründeki büyümenin devam edebilmesi için kaynaklardan en iyi şekilde yararlanmak gerekmektedir. Balık çiftliklerindeki yem israfının önüne geçmek için yemden en iyi şekilde yararlanmayı sağlamak ve birim alanda birim zamanda ve birim yemle en iyi büyümeyi sağlamak için bu tarz çalışmalara ihtiyaç duyulmaktadır.

Bu çalışmada alabalık yemine yapılan Taurin ilavesi ile balıkların pazar boyuna erken ulaşması ya da aynı süre içerisinde daha iyi canlı ağırlık kazancı elde etmesi hedeflenmiştir.

Taurin'inin balık yemlerinde kullanım olanakları üzerine çok fazla çalışma bulunmamaktadır. Daha önce yapılan bir çalışmada ortalama 0,95 gramlık şabut yavrularının büyüme parametreleri üzerine etkileri araştırılmış ve olumlu sonuçlar alınmıştır (Ateş, 2009). Balık yemlerine ilave edilen eklentilerin her boy balık grubu üzerine aynı etkiyi yapmayacağını düşünerek, daha büyük boydaki şabutlar üzerinde nasıl etki yapacağını görmek için böyle bir çalışma planlanmıştır. Ayrıca bu çalışmada Şabut balığının besin içeriği üzerine etkileri de araştırılmıştır.

MATERYAL VE YÖNTEM

Deneme Çukurova Üniversitesi Su Ürünleri Fakültesi, Dr. Nazmi Tekelioğlu Tatlısu Ürünleri Üretim ve Araştırma İstasyonu'nda, kanal suyu ve yer altı suyu kullanılarak, fiber tanklarda yürütülmüştür.

Deneme, Kontrol grubu (G1) dahil toplamda 4 gruptan oluşmaktadır. Deneme grupları Çizelge 1'de gösterilmiştir.

Çizelge 1. Deneme grupları taurin yem oranları

Gruplar	Yeme eklenen Taurin Oranı
Kontrol Grubu	1 kilogram yemde %0 taurin
Grup 1	1 kilogram yemde % 0,5 oranında taurin (5g)
Grup 2	1 kilogram yemde % 1 oranında taurin (10g)
Grup 3	1 kilogram yemde % 1,5 oranında taurin (15g)

Her bir grup 3 tekerrürden oluşmaktadır. Araştırmanın yapılacağı fiber tanklar 500 litre hacime sahiptir. Tekerrürler gruplara dağıtılırken aynı gruptaki tekerrürlerin yan yana gelmemesine dikkat edilmiş, yine her grubun tekerrürlerin yerleri gün ışığı geldiği nokta vb.

çevresel faktörlerden eşit şekilde yararlanacakları biçimde yerleştirilmiştir. Su kaynağı olarak kanal suyu ve yer altı suyu kullanılmıştır.

Ortalamaları 2,28 gramlık Şabut yavruları, DSİ Şanlıurfa 15. Bölge Müdürlüğü'nden getirilmiştir. Deneme ünitesine plastik kaplarda getirilen Şabut yavruları araştırmanın yapılacağı 25 ^{o}C deki su sıcaklığına ve 8,2 ppm oksijen seviyesine sahip tanklarda 1 saat süreyle alıştırılarak stoklanmıştır. Deneme tanklarına alınan Şabut yavruları bu işlemden 2 gün sonra günlük olarak sabah 09:00 ve akşam 16:00 saatlerinde yemlenerek deneme başlangıcı sağlanmıştır. Her 15 günde bir ara ölçüm yapılarak balıkların 2 haftalık büyüme performanslarına bakılmıştır. Ara ölçümlerdeki veriler her bireyin tek tek tartılmasıyla elde edilmiştir. Denemede granül sazan yemi kullanılmıştır ve yemin besin içeriği çizelge 2'de belirtilmiştir.

Çizelge 2. Denemede Kullanılan Sazan Yeminin Besin İçeriği

Yem	Kuru madde	Kül	Protein	Lipid
Kontrol Grubu	91,6965±0,19	8,7106±0,10	33,0222±0,87	9,3123±0,91
G1	89,3996±0,11	8,4021±0,12	33,7707±0,92	8,9283±0,16
G2	88,1569±0,15	8,4064±0,00	33,3416±0,00	9,3342±0,09
G3	88,7311±0,15	8,4252±0,06	33,4045±1,03	9,3762±0,26

Taurinin yeme karıştırılması işlemi Göçmen ve Dikel (2019)'da belirtilen bilgilerden yararlanılarak yapılmıştır.

Analizler

Deneme sonunda büyüme parametrileri ve yem tüketimi ile ilgili yapılan hesaplamalar aşağıdaki gibidir.

- Canlı Ağırlık Kazancı (%)= (Final ağırlığı – Başlangıç ağırlığı)$^{-1}$ x 100
- Ekonomik Çevirim İndeksi: EÇİ= Yem Fiyatı(S/kg) x YDO
- GAYM Günlük Alınan Yem Miktarı = Tüketilen Yem / Gün / Birey Sayısı
- Günlük Canlı Ağırlık Kazancı= (Final ağırlığı - Başlangıç ağırlığı) x gün^{-1}
- Oransal Ağırlık Artışı= [(Final ağırlığı) - (Başlangıç ağırlığı)] x (Başlangıç ağırlığı)$^{-1}$ x 100
- Spesifik Büyüme Oranı: SBO (%g gün-1)= [Ln(final ağırlığı) – Ln (başlangıç ağırlığı)] x (gün^{-1}) x 100
- Yaşama Oranı (YO) = (Deneme sonundaki balık sayısı) x (Deneme başındaki balık sayısı)$^{-1}$ x 100
- Yem Değerlendirme Oranı (YDO)= (Tüketilen yem miktarı) / (Canlı ağırlık kazancı)

- Ekonomik Dönüşüm Oranı: ECR= Yem Fiyatı(USD/kg) x FCR
- Yem Fiyatı 1,15 USD olarak kabul edilmiştir.
- Ekonomik Yarar İndeksi: EPİ= (Final ağ.(kg/balık) x Balık Fiyatı(USD/kg) - ECR
- (USD/kg) x Canlı ağırlık Kazancı (kg)
- Balık Fiyatı (USD/kg) 1,5 olarak alınmıştır.

İstatistik Hesaplamaları

60 günlük deneme periyodu sonlandığında elde edilen veriler SPSS istatistik programında one-way ANOVA (tek yönlü varyans analizi) ile analiz edilmiştir. Ortalamalar ve veriler arasındaki farklılıklar 0.05 önem seviyesinde test edilmiştir. Duncan Testi yapılarak hangi grupların birbirinden farklı olduğu belirlenmiştir.

BULGULAR VE TARTIŞMA

Bulgular

Balıkların Büyüme Performansı

Deneme 60 günlük besleme sonunda şabut yavrularının farklı dozlardaki taurin katkısı sağlanmış yemlerle göstermiş oldukları büyüme performansları Çizelge 3'de verilmiştir.

Çizelge 3. 60 Günlük Besleme Periyodu Sonrası Şabut yavrularının Büyüme Parametreleri

	Kontrol	G1	G2	G3
Başlangıç ağırlığı g)	2,28±0,02	2,28±0,01	2,28±0,01	2,28±0,00
Final Ağırlığı (g)	3,11±0,8 ^{ab}	3,05±0,09 ^{b}	3,25±0,06 ^{a}	3,14±0,14 ^{ab}
Ağırlık kazancı g)	0,82±009 ^{ab}	0,76±0,08 ^{b}	0,97±0,06 ^{a}	0,85±0,14 ^{ab}
Günlük ağırlık kazan kazancı (g/gün)	0,01±0,0 ^{a}	0,01±0,0 ^{a}	0,02±0,0 ^{a}	0,01±0,0 ^{a}
O.Büy. (%)	36,22±3,94 ^{a}	33,44±3,24 ^{a}	42,47±2,51 ^{a}	37,22±4,16 ^{a}
SGR	0,53±0,05^{a}	0,5±0,04 ^{a}	0,61±0,03 ^{a}	0,54±0,08 ^{a}
FCR	5,2±0,34^{b}	6,27±0,43^{c}	4,65±0,86^{a}	5,52±0,72^{b}
YO	%94,00	%100,00	%100,00	%100,00
ECR	7,150±0,81^{c}	7,21±0,5^{c}	5,36±0,98^{a}	6,35±0,83^{b}
EPI	0,0041±0,0^{a}	0,0040±0,0^{a}	0,0043±0,0^{a}	0,0043±0,0^{a}

O.Büy: Oransal Büyüme, SGR: Spesifik büyüme oranı, FCR: Yem Dönüşüm Oranı, YO: Yaşama Oranı, ECR:Ekonomik Çevirim İndeksi, EPI:Ekonomik Yarar İndeksi

Canlı Ağırlık Kazancı

60 günlük deneme süresince beslenen balıklardan elde edilen verilerine göre denemede sırası ile en iyi büyüme değeri %0,10 taurin eklenen gruptan (G2) elde edilirken (0,97±0,06 g); ikinci en iyi büyüme %0,15 taurin eklenen G3 grubundan elde edilmiştir (0,85±0,14); taurin takviyesi yapılmamış olan kontrol grubuna ait büyüme değerlerinin (0,82±0,09) ise %0,05 taurin eklenen G1 (0,76±0,08) grubunun büyüme değerlerinden daha iyi olduğu gözlenmiştir (P<0,05). En düşük ve en yüksek dozda taurin takviyesi yapılarak yemlenen grupların

büyüme değerlerinin (0,76±0,08 ve 0,85±0,14) kontrol grubu (0,82±009) büyüme değeri ile benzer olduğu gözlemlenmiştir (P>0,05).

Denemenin sonundaki hesaplanan verilerden ortalama SBO değerleri sırasıyla G2'de 0.58, G3'te 0.52 ve G1'de 0.46 olarak elde edilmiştir.

Oransal Ağırlık Artışı

Şabut yavruların oransal ağırlık artışları büyükten küçüğe doğru sırasıyla G2, G3, Kontrol grubu ve G1 grubunda olmuştur. Yapılan analizlere göre gruplar arasında Oransal Büyüme açısından taurin eklenmesi önemli bir fark yaratmamıştır (P>0,05)

YDO değeri en yüksek G1'de (7,21±0,5^{c}) gözlenmiş olup, sırasıyla Kontrol grubunda (7,150±0,81), G3 (6,35±0,83) ve G2 (5,36±0,98) değerleri elde edilmiştir. En iyi yem değerlendirme G1 grubunda sağlanmıştır (P<0,05). YDO açısından taurin katkısı önemli düzeyde bir farkın oluşmasını sağlamıştır.

EPI değeri bakımından en yüksek değer G3 grubundan (0,0043±0,02) ve G2 grubundan (0,0043±0,04) sağlanırken (P<0,05) daha sonra sıra ile GK grubu olup (0,0041±0,04), ve G1 grubu (0,0040±0,03) arasında fark gözlemlenememiştir.

Deneme sonunda yaşama oranlarına bakıldığında ölümlerin sadece kontrol grubunda yaşandığı gözlenmiştir. Deneme sürecinde kontrol grubunda toplamda 4 şabut yavrusunun öldüğü gözlenmiş ve veri olarak kaydedilmiştir. Taurin ile desteklenmiş olan yemlerle beslenen balık gruplarında herhangi bir ölüm olayı gözlenmemiş olup, yaşama oranı %100 olarak kaydedilmiş ve kontrol grubunda ise yaşama oranı %96±4 olarak hesaplanmış ve kayıtlara geçmiştir (P>0,05).

TARTIŞMA

Canlı ağırlık artışı, balıklara belirli bir süre verilen yemin etkili bir şekilde kullanılıp kullanılmadığını gösteren iyi bir büyüme indeksidir. Başlangıç ağırlıkları ortalama 2,28 g olan deneme gruplarımızın deneme sonu ağırlıkları incelendiğinde %1,0 taurin ilave edilen yemle beslenen grup ile diğer gruplar arasında canlı ağırlık kazancı bakımından istatistiksel farklılıklar gözlenmiştir. Denememizde en iyi büyüme performansını yemlerine % 1,0 oranda taurin ilave edilen 2. grup göstermiştir. %1,5 - %0,0 - %0,5 taurin ilave edilen gruplar sırasıyla en iyi büyüme performansına sahip olan gruplar olmuştur. Yeme farklı sevilerde taurin eklenmesi ile balıklardan farklı sonuçlar elde edilmiştir. Denemizde 3 farklı seviyede taurin eklenirken en ekonomik yetiştiriciliğin %1,0 oranında ekleme yapılan gruptan sağlanması birçok araştırmacının farklı türler üzerine bulduğu sonuçlara benzer bulunmuştur. Mercan balığı yetiştiriciliğinde en iyi büyüme oranları, yem verimi, vücuttaki taurin ve fizyolojik koşullar için taurin takviyesinin % 0.5-1 civarında yapılmasının gerekli olduğunu göstermiştir (Chatzifotis ve ark 2008). Yemlerine % 0.2 seviyesinde taurin eklenerek yapılan yemlerle beslenen trança (*Dentex dentex*) yavrularının büyüme hızlarının, yem değerlendirme oranlarının ve lipid metabolizmalarının iyileştiği görülmüştür (Chatzifotis ve ark. 2008). Taurin takviyesinin etkileri Avrupa deniz levreği (*Dicentrarchus labrax*) larvalarının büyüme performansı üzerine (Brotons-Martinez ve ark. 2004) ve SBM (Soya Fasulyesi Unu) bazlı

yemlerle beslenen genç bireylerin (Kotzamanis ve ark. 2012) büyüme değerleri değerlendirilmiştir. Beslenme faaliyeti ve büyüme oranları, artan diyet taurin takviyesi ile geliştirilmiştir. Bu sonuçlar, deniz levreği yavru diyetlerinin % 0,2 oranında taurin gerektirdiğini, yavru balıkların ise optimum performans için % 1 oranında taurin gereksinim duyduğu ortaya koyulmuştur. Göçmen ve Dikel (2019) yaptıkları çalışmada melez tilapia yavrularının (*Oreochromis niloticus x O.aureus*) farklı oranlarda taurin desteğiyle 90 gün boyunca beslenmesi sonunda, en yüksek seviyede (15g/kg) taurin takviyeli grubun balıklarının, diğer bütün gruplardaki balıklarla karşılaştırıldığında daha yüksek canlı ağırlık kazancına ulaştıkları görülmüştür. Bununla birlikte, Al-Feky ve ark (2016) yaptıkları çalışmada Nil tilapiası yavru ve juvenillerine büyüme aşamalarında taurin destekli yemle besleme gerçekleştirilmişlerdir. Diyet taurinin tatlı su balıklarının larva performansı üzerindeki etkileri, özellikle Nil tilapia larvaları üzerindeki çok sınırlıdır. Bu çalışmada taurin içermeyen bir diyet, düşük büyüme performansı ile sonuçlanırken, 10 g/kg diyet taurin en iyi büyüme oranları ve yem verimliliği ile sonuçlandığı belirtilmiştir. Ancak, ikinci dereceden regresyon analizleri, maksimum larva performansının yaklaşık 9.7 g/kg diyet taurin olarak gerçekleştiğini göstermiştir. Bu değer Goncalves ve ark., (2011) tarafından bildirilenden biraz daha yüksek bulunmuştur.

Al Feky ve ark., (2016), Nil tilapia yavruları üzerine yaptıkları çalışmada en iyi büyümenin %1 taurin eklenen grupta olduğunu bunu %0,5 ve %1,5'lik taurin eklenen grupların izlediğini bildirmiştir. Kontrol grubu bireylerinin büyümesi en geride kalan grup olmuştur. Denememizde ise bunun ötesinde bazı sonuçlar ortaya çıkmıştır. Denememizde kontrol grubu bireyleri daha iyi yem değerlendirmelerine karşın büyüme olarak en geride olamamakla beraber en yüksek (%1,0) grubun hemen ardından ikinci sırada yer almışlardır. Bulunan bu sonuç Göçmen ve Dikel., (2019)'nin ulaştığı sonuçla desteklenmektedir. Zira o çalışmada da kontrol grubu bireylerinin daha düşük FCR'na ulaştıkları bildirilmiştir. Bunların tersine El Feky ve ark., (2016) %1 taurin ekledikleri gruptan en iyi FCR sonuçları alınırken mevcut çalışmada ise en iyi FCR taurin eklenmeyen grupla ulaşılmıştır. Yaşama oranı bakımından da Al Feky ve ark., (2016)'nın bulgularının aksine kontrol grubundan kayıplar olmasına karşın diğer muamelelerden kayıp yaşanmamıştır.

Deneme sürecinde elde ettiğimiz büyüme parametrelerine bakıldığında taurin'in şabut yavrularının büyümesi üzerine etkilerinin kaynaklara paralel olduğu görülmektedir. %1,00 taurin eklenen grubun 60 gün sonunda diğer gruplara göre daha fazla büyüdüğü gözlenmiştir.

Yapılan ekonomik analizler sonucunda taurin ile desteklenmiş olan yemlerle beslenen grupların nispetende olsa daha düşük maliyetlerle üretim olanağı verebileceği görülmektedir. Ancak denememizde özellikle yemine taurin eklenmemiş olan grup ile EÇO azalmıştır. Bunun anlamı, şabut yavrularında taurin desteği olmaksızın daha düşük bir maliyetle yem çevirimi elde edilmiştir. Yetiştiricilikte yem çevirim oranının düşmesi için zaman zaman yemlere yapılan katkılar ile başarı elde edilmiştir (Dikel ve ark 2010; Dikel ve Yabacı 2016).

Deneme elde edilen sonuçlara göre şabut yavrularının vücut nem içeriği ve külü, artan diyet taurin ile değişmezken, vücut kompozisyonları diyet taurin takviyesi ile protein ve yağ

açısından önemli ölçüde değişmiştir. Benzer sonuçlar Al Feky ve ark (2016), tarafından da beyan edilmiştir.

Taurinin yavrularda sağ kalım üzerine etkisi açısından değerlendirme yapmak için bakıldığında denememizde elde ettiğimiz verilere göre kontrol grubunda ölüm gözlenirken taurin katkılı yemlerle beslenen gruplarda ölüm görülmemiştir. Bunun aksine Al Feky ve ark (2016) Nil tilapialarında larva sağ kalımın, 10 kg $^{-1}$ 'e kadar olan taurin takviyesinden önemli ölçüde etkilenmediğini hatta taurinin 15 kg $^{-1}$ 'e yükseltilmesi ile balık sağ kalımında keskin bir düşüşe yol açtığını bildirmişlerdir.

Yapılan bu çalışma ile taurinin şabut üreticisine yetiştiricilik sürecinde hem büyüme parametrelerinde hem de üretim maliyetinde yapacağı destek incelenmiştir. Günümüzde balık üretim sektöründe yoğunlukla uygulanan yem katkı maddeleri başlığı altında birçok balık türü için ciddi bir biçimde uygulanmakta olan ek katkı maddeleri şabut beslemede oluşturacağı etki ve elde edilmesi olası kazanım ilgi konusu olmuştur. Bu noktadan çıkılarak yapılmış araştırmaların da ışığı altında belli dozlarda toz taurinin şabut yavrularının Çukurova'da belli bir pozitif katkı yarattığı ve ekonomik açıdan da önerilebilir katkılar yarattığı sonucuna ulaşılmıştır. Elde edilen verilere göre, en iyi büyüme değerleri % 1 düzeyinde taurin eklenen gruptan elde edilmiştir. Bu açıdan bakıldığında yeme 10g/kg taurin eklenmesi şabut yavrularının büyütülmesi esnasında yem sektörü için önerilebilir bir uygulama olarak belirtilebilir. Yavruların canlı kalma yüzdelerinin yüksek tutulması konusunda bu çalışma şunu göstermiştir ki yeme taurin eklenmesi şabut yavrularının canlı kalma oranlarını pozitif yönde etkilemiştir. Bu nedenle deneme sonu elde edilen veriler bu açıdan da değerli katkılara sahiptir. Büyüme hızı açısından değerlendirme yapıldığında, Çukurova bölgesi gibi şabut üretim periyodunun kısıtlı olduğu bölgelerde hayli önemli sonuçlar elde edilmiştir. Zira kısa bir sürede pazar boyuna ulaşmak için büyük boyda semirtmeye geçilmesi daha başarılı sonuçlara olanak sağlamaktadır. Araştırma sonuçları tümüyle dikkate alınacak olursa, şabut yavrularının yemlerine yapılacak olan taurin desteği şabut yavrularının büyüme performansında pozitif etki yaratmış olup önerilebilir ve uygun taurin desteğinin şabut yavruları için % 1 olduğu söylenebilir. Şabut yavruların yaşam oranının yüksek tutulabilmesi için taurin takviyesi önerilebilir. Şabut yavrularının yemine taurin eklenerek pazar boyuna kadar büyütülmeleri önerilebilir. Bu şekilde beslenerek büyütülen şabutların karkas kalitesi ve etinde oluşacak değişimlerin incelenmesi önerilebilir. Taurin destekli yemle yapılan yetiştiricilik sonrası üretilen şabut etinin saklama koşullarında vereceği olası avantajlar ve değişiklikler incelenebilir. Denemede 15'er gün arayla tartılan şabut yavrularının her tartım işleminden sonra ilk bir hafta yem almadıkları gözlenmiş olup, şabutların stresten uzun süre çıkamadıkları ve yetiştiricilikte rahatsız edilmemeleri önerilebilir.

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Assesstment of Asthma-Associated miRNAs in Children with Rhinovirus Respiratory Infections

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Abstract

Aim: Asthma is one of the major health problems all over the world. In fact, approximately two-thirds of asthma exacerbations are associated with human rhinovirus (HRV). MicroRNAs (miRNAs) are small single-stranded, non-coding RNA molecules 22-25 nucleotides in length. It has been reported that miRNA expression levels may be associated with the pathogenesis of diseases through the regulation of the production of target proteins. Expression changes of several miRNAs have also been found to play a role in the development and/or improvement of asthma. Still, relatively little is known about the role of miRNAs in asthma. We aimed to investigate serum levels of five asthma-associated miRNAs in children with lower respiratory tract infection (LRTI) caused by HRV compared to age-matched healthy controls.

Materials and Method: Serum samples were collected from the 37 pediatric patients, aged 0-3 years, with LRTI proven to be caused by HRV using RT-PCR assay (FTD Respiratory pathogens 21, Fast Track Diagnostics, Luxembourg) and from the 30 age-matched healthy controls. miRNA let-7a-5p, miR-9-5p, and miR-155-5p were investigated from the serum samples by using RT-PCR assay (RTA miRNA Kit, Kocaeli, Turkey). miRNA serum expression levels were analyzed by the $2^{(-\Delta\Delta CT)}$ method.

Results and Conclusion: In the present study, we found that let-7a-5p, miR-9-5p and miR-155-5p levels were significantly increased, while miR-146a-5p and miR-19-3p levels were significantly decreased in the sera of children with HRV infection compared to the age-matched control group. Assessment of serum expression changes of asthma-associated miRNAs in children with respiratory tract infections caused by HRV is important for understanding the effect of HRV on the pathogenesis of asthma through miRNA expression changes. On the other hand, longitudinal studies based on clinical observation are needed to determine the association of HRV infection with the development of asthma and miRNA-induced epigenetic effects.

Keywords: Human rhinovirus, asthma, miRNA let-7a-5p, miR-9-5p, miR-146a-5p, miR-19-3p, miR-155-5p

INTRODUCTION

Asthma is the most common chronic inflammatory airway disease. Main manifestations of asthma are coughing and wheezing due to airway narrowing. In addition to allergens, infections with respiratory tract viruses are important triggers for exacerbation of asthma symptoms (Lambrecht, & Hammad, 2015). Human rhinoviruses (HRVs) are responsible for asthma exacerbations in 50-80% cases (Nakagome et al., 2014). HRVs are positive-strand RNA viruses that infect the upper and lower airways and may cause severe lower respirtory tract infections in infants and early childhood (Kennedy et al., 2012). HRV infection and replication in epithelial cells activate innate immune responses and induce expression of growth factors and chemokines, which leads to recruitment and activation of granulocytes, including neutrophils, destruction of normal airway tissue, and development of respiratory symptoms (Message & Johnston 2001). miRNAs are short non-coding RNAs that modulate various biological processes through post-transcriptional regulation of gene expression (Zhang & Farwell, 2008). Translation of mRNA is inhibited or mRNA degradation is triggered by miRNA binding proteins (Quévillon & Simard, 2019). miRNAs are capable of regulating immune responses in various conditions (Trobaugh & Klimstra, 2017). Several miRNAs have also been found to play a role in the development and improvement of asthma (Rebane & Akdis, 2014).

The aim of the present study is to investigate alterations in serum levels of five asthma-associated miRNAs, miR-146a, miR-19, let-7a, miR-9, and miR-155 in children with lower respiratory infection caused by HRV, compared to age-matched healthy controls.

MATERIALS AND METHOD

Serum samples were collected from 37 pediatric patients with HRV infection, aged 0-3 years, simultaneously with bronchoalveolar lavage fluid samples in Pediatric Pulmonology clinic in Meram Faculty of Medicine in 2017. Serum samples were collected from 30 age-matched healthy controls who attended to pediatrics for a routine follow-up. miR-146a, miR-19, let-7a, miR-9, and miR-155 were investigated from the serum samples by using RT-PCR assay (Applied Biological Materials Inc., Richmond, Canada) after miRNA extraction (RTA miRNA Kit, Kocaeli, Turkey). miRNA serum expression levels were analyzed by the $2^{(-\Delta\Delta CT)}$ method. U6 was used as the endogenous control. The difference between the groups was analyzed with one-way ANOVA test in SPSS Statistics 22 program. A p-value less than 0.05 was accepted as statistically significant.

An approval was obtained from the Ethics Committee of Necmettin Erbakan University, Meram Faculty of Medicine (decision number:2017/1004). This study is supported by Scientific Activities Support Program of Necmettin Erbakan University (Project Number:171218008).

RESULTS

In the present study, we found that let-7a-5p, miR-9-5p and miR-155-5p levels were significantly increased, while miR-146a-5p and miR-19-3p levels were significantly decreased in the sera of children with HRV infection compared to the age-matched control group. The serum levels of each miRNA are shown in Table 1.

Table 1. Mean $2^{-\Delta\Delta CT}$ levels of miRNAs

miRNA	Mean $2^{-\Delta\Delta CT}$	SS	%95 CI	p
U6*	1,000	0,000	(0,978; 1,022)	NA
let-7a-5p	1,04973	0,04252	(1,03015; 1,06931)	0.000*
miR-146a-5p	0,8500	0,0723	(0,8304; 0,8696)	0.000*
miR-19-3p	0,5900	0,0756	(0,5704; 0,6096)	0.000*
miR-9-5p	1,15000	0,04534	(1,13042; 1,16958)	0.000*
miR-155-5p	1,7600	0,0799	(1,7404; 1,7796)	0.000*

DISCUSSION

Risk factors for childhood asthma are still poorly understood. Environmental exposures during childhood, as well as airway obstruction that develops during this period, are major contributors in the development of persistent asthma in children. Genetic susceptibility is one of the contributing factors, as well (Panettieri et al., 2008). The reason why we chose children under the age of three was because of the long term airway remodeling and the risk for the development of asthma are mostly enhanced in the first three years of life (Malmström et al., 2015).

In experimental models, HRV infection has been shown to cause airway hyperreactivity (Jakiela et al., 2021). The immune response derived from the Th2 cells seems to be important in the pathophysiology of asthma (Athari et al., 2019). The infiltration of eosinophils and T lymphocytes is the main characteristics of the airway during an asthma exacerbation. The type 2 inflammatory response with interleukin (IL)-4, IL-5 and IL-13 (allergic cytokines) are the key players in asthma. Whereas, IFN-γ, a type 1 inflammatory cytokine, is crucial in an antiviral immune response to reduce related immune damage (Athari, 2019). The pathophisiology of asthma is associatd with the dysregulation of signaling pathways that control the IFN production rather than an IFN deficiency (Ather et al., 2011). The immune response to viral infection may be altered by shifting the balance of T-cell cytokine expression from type 1 to type 2, resulting in allergic inflammation (Athari, 2019. Virus-induced interferon (IFN)-β and IFN-λ production have been shown to be significantly reduced in asthmatic patients (Ather et al., 2011).

The pathophysiology of asthma is assumed to be linked to the activation of the NF-KB signaling pathway in airway epithelial cells (Athari, 2019). miR-146a and miR-155 inhibit NF-κB and IRF pathways (Oglesby et al., 2010). miR-155 induces Th2-mediated eosinophilic inflammation in the lung (Malmhäll et al., 2014). Also, miR-155 deficient mice have been shown to display immunodeficiency and substantial airway remodelling (Rodriguez et al., 2007). Furthermore, miR-155 transfection in human bronchial epithelial-derived cell line suppresses HRV infection (Bondanese et al., 2014). We found the miR-155-5p serum levels were significantly increased and miR-146-5p serum levels were significantly decreased in HRV-infected children.

Airway smooth muscle cells are the major contributer to the airway wall thickness via proliferation, cell migration, and deposition of extracellular matrix and accompany protein arginine methyltransferase 1 (PRMT1) in airway remodeling (Sun et al., 2017). Decreased miR-19a levels have been found to be associated with the expression of methyltransferase 1 enzyme in asthmatic respiratory smooth muscle cells and air way remodeling (Sun et al., 2017). Sun et al., (2017) reported that reduced miR-19a expression induces upregulation of ERK1/2 MAPK, STAT1, and PRMT1 in turn, causing airway remodeling. In the present study, we found that miR-19a serum levels in HRV-infected children were significantly decreased compared to the healthy controls.

Other investigators have reported several other miRNAs associated with the inflammatory processes in asthma. miR-21, miR-126, and miR-145 are important regulators of recruitment of eosinophils and neutrophils into the airway and reduced mucus hypersecretion (Foster et al., 2017).

The let-7 family is involved in pro-inflammatory cytokine production, inhibiting IL-13 expression which is characteristic for high-Th2 cells-asthma type (Zhang et al., 2019; Rijavec et al., 2014). miR-9 was also demonstrated to be increased in sputum from neutrophilic asthma, but not in eosinophilic asthma (Li et al., 2015).).

In conclusion, this is one of the rare attempts to investigate asthma associated miRNAs in pediatric patients with HRV infection to address the susceptibility of asthma development. Our findings suggest that dysregulation in the serum levels of asthma-associated miR-146a, miR-19, let-7a, miR-9, and miR-155 may be associated with the HRV replication and virus-induced asthma development. On the other hand, longitudinal studies based on clinical observation are needed to determine the association of HRV infection with the development of asthma and miRNA-induced epigenetic effects.

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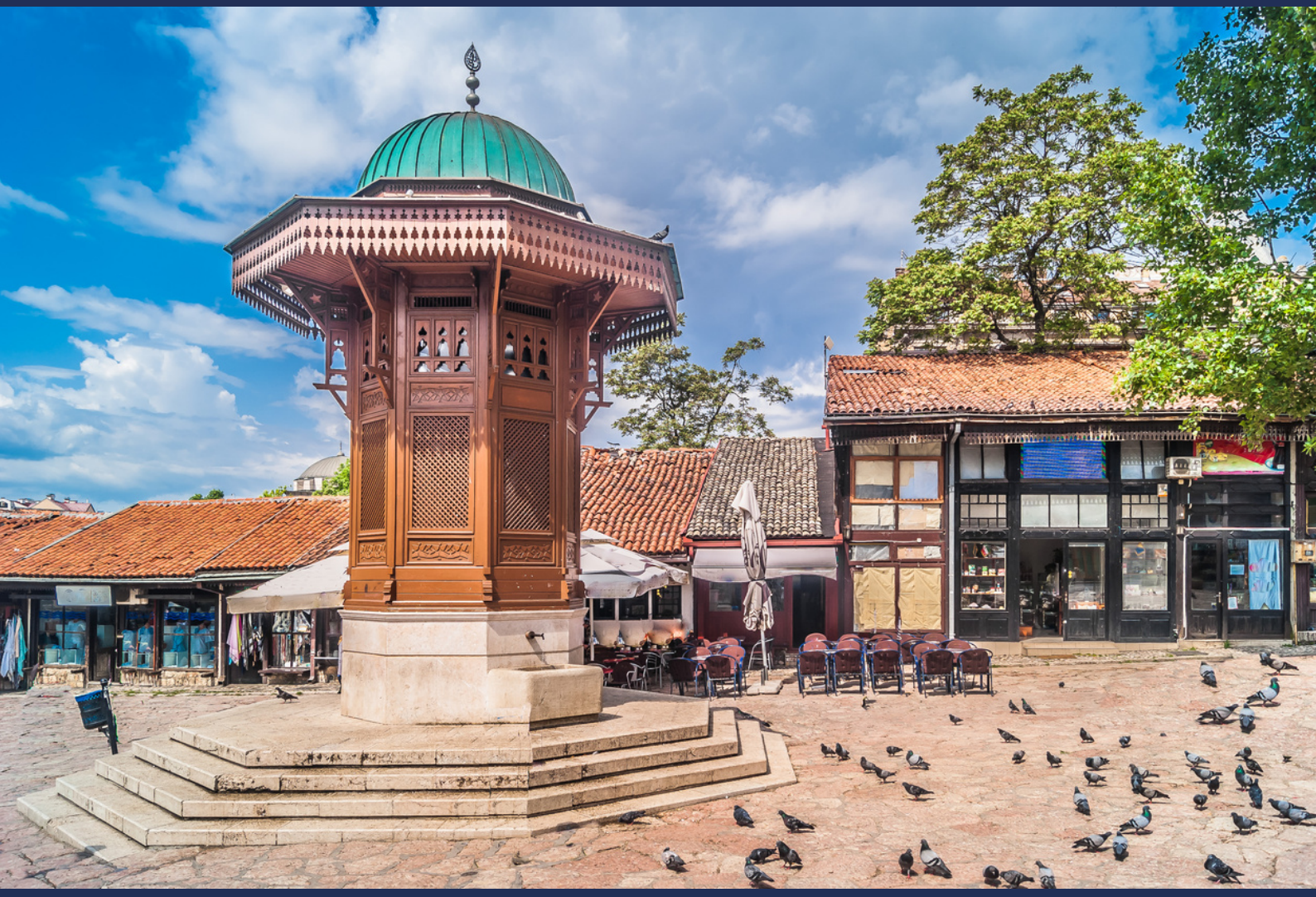

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